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Supplemental Information

The Leucokinin Pathway and Its Neurons Regulate Meal Size in *Drosophila*

Bader Al-Anzi, Elena Armand, Paul Nagamei, Margaret Olszewski, Viveca Sapin, Christopher Waters, Kai Zinn, Robert J. Wyman, and Seymour Benzer

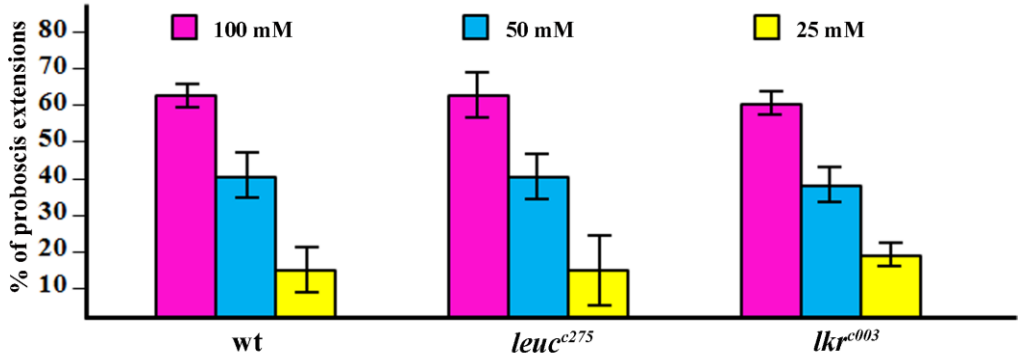

Figure S1. Mutations in the Leucokinin Pathway Do Not Produce Defects in Gustatory Detection of Different Concentrations of Sucrose (Related to Figure 2)

Wild-type 24 hour starved C-S flies extend their proboscis when their feet are contacted with different concentrations of sugars. The extension probability decreases with lowered concentrations. *leuc*^{c275} and *lkr*^{coo3} mutants do not show any difference in proboscis extension probability from wild-type flies. Concentrations of sucrose used are shown at top. n = 25 flies per experiment. Asterisks denote statistical significance: *p < 0.05; **p < 0.01; ***p < 0.005; Student's t test.

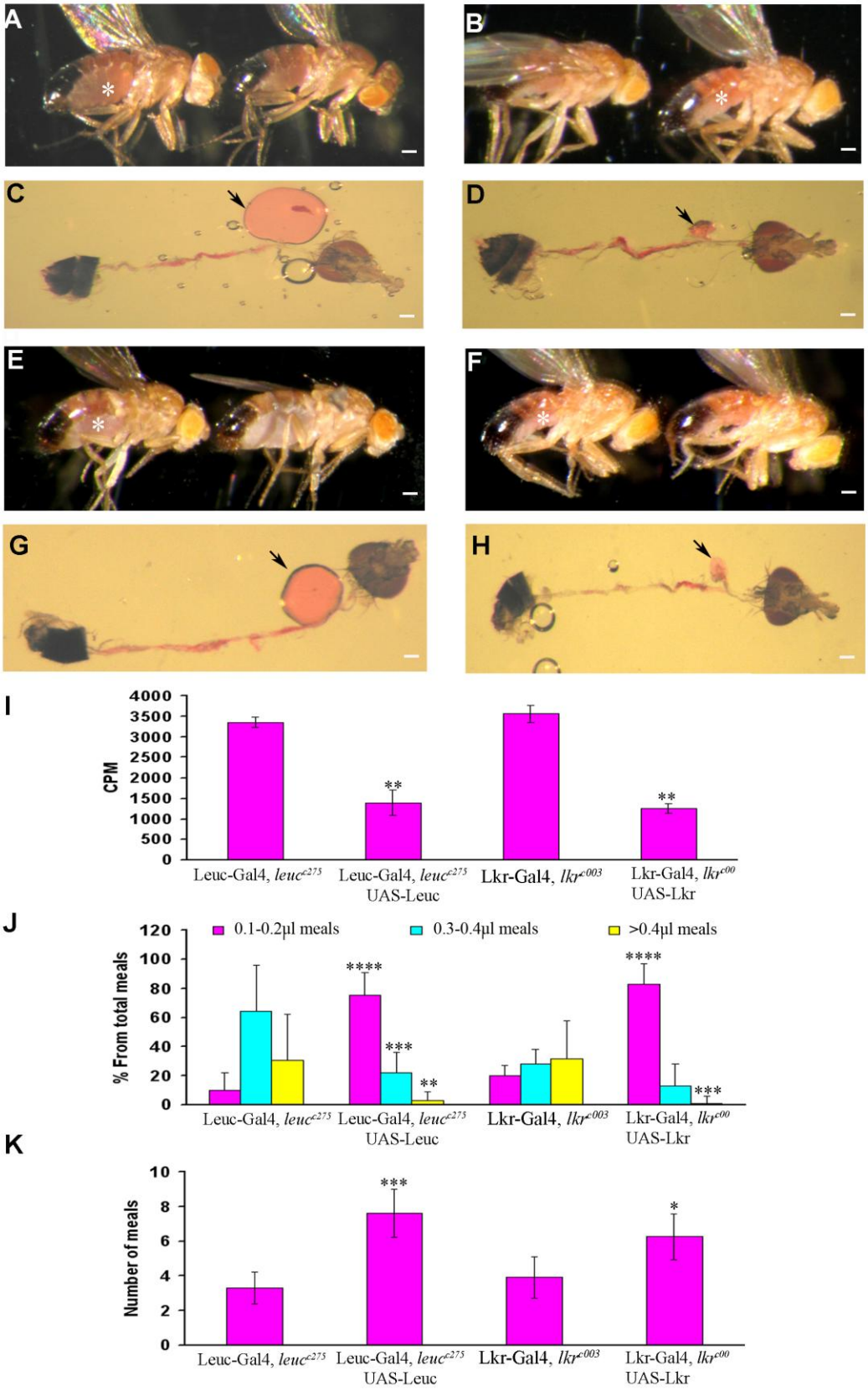
A
B
C
D
E
F
G
H
I
CPM
4000
3500
3000
2500
2000
1500
1000
500
0
**
**
Leuc-Gal4, leuc^c275
Leuc-Gal4, leuc^c275
UAS-Leuc
Lkr-Gal4, lkr^c003
Lkr-Gal4, lkr^c00
UAS-Lkr
J
0.1-0.2µl meals
0.3-0.4µl meals
>0.4µl meals
% From total meals
120
100
80
60
40
20
0
****
***
**
****
***
Leuc-Gal4, leuc^c275
Leuc-Gal4, leuc^c275
UAS-Leuc
Lkr-Gal4, lkr^c003
Lkr-Gal4, lkr^c00
UAS-Lkr
K
Number of meals
10
8
6
4
2
0
***
*
Leuc-Gal4, leuc^c275
Leuc-Gal4, leuc^c275
UAS-Leuc
Lkr-Gal4, lkr^c003
Lkr-Gal4, lkr^c00
UAS-Lkr

Figure S2.

Figure S2. **Regulation of Meal Size by the Leucokinin Pathway Is Mediated by the Leuc-Gal4 and Lkr-Gal4 Neurons (Related to Figure 5)**

Expression of UAS-Leuc using the Leuc-Gal4 driver rescues the *leuc*^{c273} flies' crop bloating defect in the two-dye assay (B and D), the poststarvation 14C-leucine food intake defect (I), and the meal size and frequency defect in the single fly café assay (J and K, respectively). No rescue of these parameters is observed in control mutant *leuc*^{c273} flies that carry only the leuc-Gal4 driver (A-C, and I-J). Expression of UAS-Lkr using the Lkr-Gal4 driver in *lkr*^{c003} flies rescues the crop bloating defect in the two-dye assay (F and H), the 14C-leucine food intake defect (I), and the meal size and frequency defect in the single fly café assay (J and K, respectively). No rescue of these parameters is observed in control mutant *lkr*^{c003} flies that carry only the Lkr-Gal4 driver (A, C, and I-J). For *leuc*^{c273}/UAS-Leuc, and *lkr*^{c003} /UAS-Lkr controls see Figures 1 and 2. White scale bar represents 200 μm. Error bars are standard deviation of 5-8 different replicates for a given genotype in A and K, and of 20-25 single fly analyses in (B)-(J). Asterisks denote T-test statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0005$.

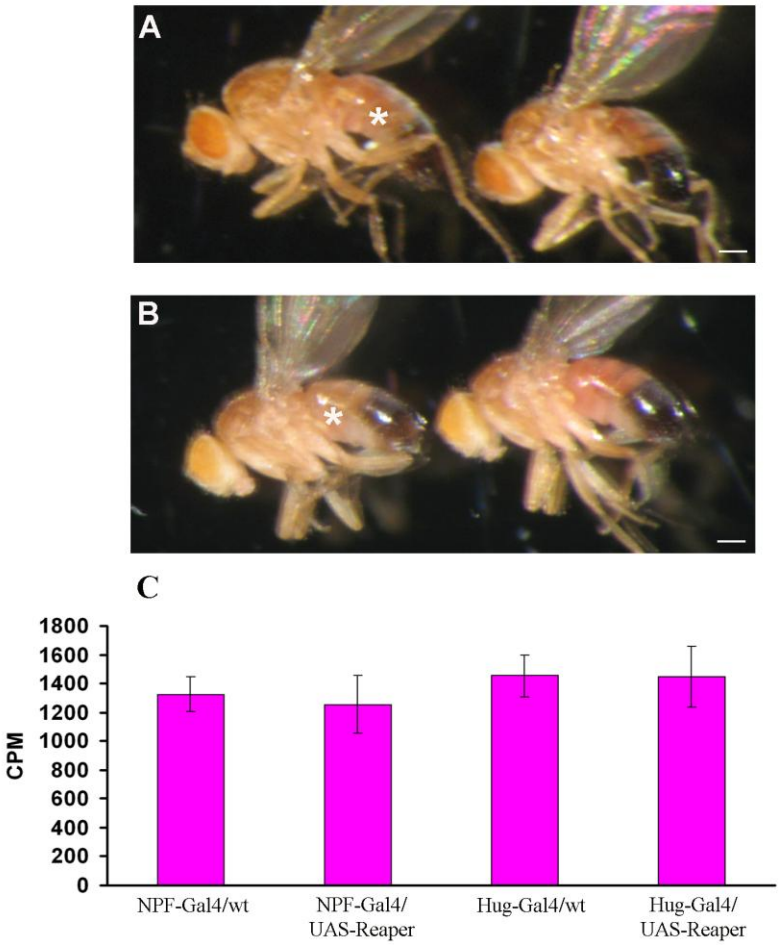

Figure S3. **Ablation of Neurons Expressing NPF-Gal4 (A and C) and Hug-Gal4 (B and C) by Transgenic Expression of UAS-Reaper Does Not Produce Feeding Behavior Defects when Flies Are Subjected to the Two-Dye (A and B) or 14C-Leucine Assays (Related to Figure 5)**

White scale bar represents 200 μm. Error bars are standard deviation of 5-8 different replicates for a given genotype. Asterisks denote t test statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0005$.